Saskatchewan
Agriculture, Food
and Rural
Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20000229

DEVELOPING CALIBRATION EQUATIONS FOR NEAR INFRARED SPECTRUM AND CHEMICAL COMPOSITION OF FEED PEAS

Funded by:
The Agriculture Development Fund

February 2003
Prepared by: University of Saskatchewan

Final Report

Developing calibration equations for near infrared spectrum and chemical composition of feed peas

Tom Warkentin, Vern Racz and Gene Arganosa
December 18, 2002
Project: 20000229

(a) Abstract/Summary

The goal of this project was to extend an existing database on chemical composition and Near Infrared Spectroscopy (NIRS) spectra for varieties of field pea grown at several locations in Saskatchewan. Calibration equations were developed to predict starch, protein, acid detergent fiber, neutral detergent fiber, ether extract, and ash content of field pea seeds. Wet chemistry and NIR spectra were determined for 31 advanced breeding lines and 5 check varieties grown at four locations in each of 2000 and 2001. Wet chemistry revealed substantial ranges in mean values for all chemical parameters assessed. Several advanced lines were superior to the best check variety in two or more key traits (crude protein, ADF, NDF, or starch) related to feed value, without being inferior in any key trait. NIRS calibrations on ground grain samples were more reliable than calibrations using whole grain samples. Reliable NIRS calibrations, on ground pea samples, are now available

(b) Executive Summary

The protein, fibre and starch contents of field pea are the primary factors that define nutritional quality. Field pea is primarily fed to pigs and chickens and these species do not make good use of dietary fibre. Thus, field peas that are high in starch and protein and low in fibre have a higher nutrient density and are more desirable for use in feeds than those with high fibre content.

The near infrared spectra of biological materials such as field peas are dependent upon the chemical composition of the material. It is possible to calibrate Near Infrared Spectroscopy (NIRS) instruments to chemical and even biological characteristics of feed ingredients. Calibration equations can then be developed to accurately assess the chemical or biological characteristics of a feed ingredient from the NIRS spectrum of the sample. Industrial application of NIRS calibrations is currently used to predict protein and starch composition of materials rapidly and at low cost.

The existing database was developed in a collaborative project (1999-2000) between the Prairie Feed Resource Centre (PFRC) and the Crop Development Centre (CDC), funded by the Saskatchewan Pulse Growers (see report attached). In the first project, 125 field pea samples were analyzed by wet chemistry for starch, protein, acid detergent fiber, neutral detergent fiber, ether extract, ash, and moisture. These whole grain samples were also scanned by NIRS, and correlation equations were developed between the spectra and the chemical parameters. Calibration equations were developed with r-squared values of 0.95 for protein, 0.76 for acid detergent fiber, 0.75 for starch, 0.54 for neutral detergent fiber and 0.49 for ether extract. With the NIRS calibration equations developed from this data, it was possible to use NIRS to accurately predict the protein content of field pea samples. The calibration equations for acid detergent fibre and starch are less accurate. The equations for neutral detergent fibre and ether extract were not reliable.

The objectives of the current project were (1) to implement a system of rapid, low cost, NIRS methodology for selection of field pea genotypes with superior feed quality in breeding programs,

and (2) to implement this system for use by industry to effectively assess the chemical composition of field pea samples for animal feeding.

The CDC supplied 288 genetically diverse samples of field pea for analysis by wet chemistry and NIR. These samples consisted of 31 advanced breeding lines and 5 check varieties grown at 4 locations (Saskatoon, Elrose, Outlook, and Rosthern) during the 2000 growing season, and a second set of 32 advanced breeding lines and 4 checks grown at the same 4 locations in 2001. These samples were analysed by wet chemistry for starch, protein, acid detergent fibre, neutral detergent fibre, ash, moisture and ether extract, and NIRS spectra were collected on all samples in whole grain and ground form. Wet chemistry revealed substantial ranges in mean values for all chemical parameters assessed. Ideally, we would like to select feed pea varieties, which are high in protein and starch and low in fibre (ADF and NDF). Several advanced lines were superior to the best check variety in two or more key traits (crude protein, ADF, NDF, or starch) related to feed value, without being inferior in any key trait.

The RSQ, 1-VR and SECV statistics for the nutrient parameters in field pea, based on **whole grain scans** of the 2000 and 2001 samples, are as follows:
% Crude protein: 0.94, 0.91, 0.61
% ADF: 0.52, 0.47, 0.56
% NDF: 0.36, 0.28, 1.36
% Ether extract: 0.50, 0.30, 0.14
% Ash: 0.83, 0.67, 0.18
% Starch: 0.33, 0.14, 2.94

The RSQ, 1-VR and SECV statistics for the nutrient parameters in field pea, based on **ground grain scans** of the 2000 and 2001 samples, are as follows:
% Crude protein: 0.98, 0.93, 0.51
% ADF: 0.76, 0.61, 0.48
% NDF: 0.54, 0.34, 1.20
% Ether extract: 0.80, 0.57, 0.10
% Ash: 0.89, 0.78, 0.16
% Starch: 0.65, 0.37, 2.50

The calibrations based on ground samples will allow users to predict crude protein content, ash, and ether extract with a high degree of confidence, and to predict starch and ADF with a moderate degree of confidence.

(c) Technical Report

Background:

Field pea is an increasingly important crop in Saskatchewan with 2.75 million acres of production in 2001. The majority of Saskatchewan field pea production is used for animal feeding. In the past, breeding of field pea varieties had not focused on nutritional value for animal feeding. As such, the opportunity exists to select varieties that combine good agronomic characteristics with good nutritional value for targeted species of livestock.

The protein, fibre and starch contents of field pea are the primary factors that define nutritional quality. Field pea is primarily fed to pigs and chickens and these species do not make good use of dietary fibre. Thus, field peas that are high in starch and protein and low in fibre have a higher nutrient density and are more desirable for use in feeds than those with high fibre content.

The near infrared spectra of biological materials such as field peas are dependent upon the chemical composition of the material. It is possible to calibrate Near Infrared Spectroscopy (NIRS) instruments to chemical and even biological characteristics of feed ingredients. Calibration equations can then be developed to accurately assess the chemical or biological characteristics of a feed ingredient from the NIRS spectrum of the sample. Industrial application of NIRS calibrations is currently used to predict protein and starch composition of materials rapidly and at low cost, and in some cases, without destruction of the sample.

In the first project, 125 samples of field pea were analyzed by wet chemistry for starch, protein, acid detergent fiber, neutral detergent fiber, ether extract, ash, and moisture. Near infrared spectra were also collected for each of these samples and correlation equations were developed between the spectra and the chemical parameters. Calibration equations were developed with r-squared values of 0.95 for protein, 0.76 for acid detergent fiber, 0.75 for starch, 0.54 for neutral detergent fiber and 0.49 for ether extract. With the NIRS calibration equations developed from this data, it was possible to use NIRS to accurately predict the protein content of field pea samples. The calibration equations for the remaining constituents were less accurate. The goal of the current project was to extend the database to improve the NIRS calibration equations and increase the r-squared values. As such, further work on chemical composition and NIRS of multiple varieties of field pea should result in a complete set of calibrations that can be used by field pea breeders and industry to rapidly assess the key chemical parameters of starch, protein and fibre content.

Project Objectives:

The objectives of this research project were (1) to implement a system of rapid, low cost, NIRS methodology for selection of field pea genotypes with superior feed quality in breeding programs, and (2) to implement this system for use by industry to effectively assess the chemical composition of field pea samples for animal feeding.

Materials and Methods

The CDC supplied 144 genetically diverse samples of field pea for analysis by wet chemistry and NIR in each of 2000 and 2001. These samples consisted of 36 advanced breeding lines grown at 4 locations (Saskatoon, Elrose, Outlook, and Rosthern). These samples were analysed by wet chemistry for starch, protein, acid detergent fibre, neutral detergent fibre, ash, moisture and ether extract, and NIRS spectra were collected for each sample.

Results and Discussion

Wet chemistry analysis of field pea samples grown at four locations in 2000 are summarized in Table 1. Substantial ranges in mean values occurred for all traits measured. Several advanced lines were superior to the best check variety in two or more key traits (crude protein, ADF, NDF, or starch) related to feed value, without being inferior in any key trait. Line 635-10 was superior in crude protein, ADF, and NDF; lines 635-25, 585-1, 585-4, 643-3, 643-7, 647-18 were superior in crude protein and NDF; lines 647-14 and 585-4 were superior in crude protein and ADF. Line 655-3 was superior in NDF and starch, but inferior in crude protein.

Wet chemistry analysis of field pea samples grown at four locations in 2001 are summarized in Table 2. Substantial ranges in mean values occurred for all traits measured. Several advanced lines were similar or superior to the best check variety in two or more key traits (crude protein, ADF or starch) related to feed value, without being inferior in any key trait. Line 429-4 was superior in protein content and starch. CDC0009, CDC0010, and CDC0108 had high protein content and low ADF. CDC0103 had low ADF and high starch.

The RSQ, 1-VR and SECV statistics for the nutrient parameters in field pea, based on **whole grain scans** of the 2000 and 2001 samples, are as follows:
% Crude protein: 0.94, 0.91, 0.61
% ADF: 0.52, 0.47, 0.56
% NDF: 0.36, 0.28, 1.36
% Ether extract: 0.50, 0.30, 0.14
% Ash: 0.83, 0.67, 0.18
% Starch: 0.33, 0.14, 2.94

The RSQ, 1-VR and SECV statistics for the nutrient parameters in field pea, based on **ground grain scans** of the 2000 and 2001 samples, are as follows:
% Crude protein: 0.98, 0.93, 0.51
% ADF: 0.76, 0.61, 0.48
% NDF: 0.54, 0.34, 1.20
% Ether extract: 0.80, 0.57, 0.10
% Ash: 0.89, 0.78, 0.16
% Starch: 0.65, 0.37, 2.50

The calibrations based on ground samples will allow users to predict crude protein content, ash, and ether extract with a high degree of confidence, and to predict starch and ADF with a moderate degree of confidence.

Technology transfer. Technology transfer activities are in progress. Calibration equations are being combined with data developed by researchers at Alberta Agriculture, Food and Rural Development, Agri-Food Laboratories Branch, Edmonton. A combined database should increase reliability of the NIR calibrations. We plan to publish the results of this research, which will facilitate the use of these calibrations by other pea breeders who are developing varieties for Saskatchewan.

The PFRC is well connected with the Saskatchewan feeds industry. The primary vehicle for technology transfer will be direct communication of calibration equations to industry partners such as the Saskatchewan Wheat Pool. In addition, the results and conclusions would be given wide exposure through the publications of the PFRC. The PFRC publishes Feed News and Feed Notes, which are widely distributed within the feed industry and the associated research and development community. An article on this project will be included in these publications.

Table 1. Wet chemistry analysis, expressed on a dry weight basis, of entries in the Crop Development Centre 2000 Pea Advanced Yield Trial-03 (mean data from trials grown at Saskatoon, Rosthern, Elrose, and Outlook)

Entry	Variety	% Moisture	% Crude protein	% ADF	% NDF	% Ether extract	GE (cal/g)	% Ash	% Starch
1	CDC Mozart	8.2	23.5	8.5	14.3	1.2	4367	3.0	43.5
2	Carneval	8.2	22.9	8.5	14.4	1.3	4354	3.2	42.3
3	Alfetta	8.0	23.1	7.9	13.7	1.1	4365	3.0	42.2
4	Miami	8.0	23.4	7.5	14.1	1.1	4357	3.0	40.6
5	521-14	8.0	23.4	8.5	14.7	1.3	4363	3.1	43.4
6	Keoma	8.1	22.9	8.6	15.2	1.5	4384	3.0	41.8
7	648-7	8.2	23.3	8.3	13.1	1.2	4363	2.9	43.4
8	635-6	8.1	23.2	7.8	13.5	1.2	4345	3.1	44.1
9	635-10	8.3	23.8	7.5	12.8	1.2	4386	3.0	42.0
10	635-14	8.2	23.1	7.9	14.1	1.2	4372	3.0	43.7
11	635-15	8.2	24.2	8.6	13.7	1.2	4359	3.0	41.8
12	635-25	8.1	23.8	8.3	12.8	1.2	4382	3.1	42.0
13	585-1	8.2	25.0	7.9	13.4	1.3	4390	3.0	41.5
14	585-3	8.2	26.8	8.4	14.9	1.3	4406	3.0	41.2
15	585-4	8.2	26.0	8.3	13.4	1.1	4393	2.9	42.4
16	617-3	8.2	23.6	8.2	14.5	1.2	4380	3.0	41.3
17	679-3	8.2	22.6	8.4	15.4	1.4	4340	3.2	43.3
18	679-4	8.1	23.7	9.2	15.7	1.1	4354	3.1	42.7
19	643-3	8.1	25.6	8.3	13.5	1.1	4364	3.1	43.5
20	643-7	8.1	25.4	8.1	12.5	1.3	4386	3.1	42.4
21	453-34	8.1	22.7	8.4	13.7	1.2	4368	3.1	44.3
22	652-16	8.0	24.3	8.4	14.9	1.1	4364	3.1	41.3
23	652-18	8.2	23.2	8.2	13.9	1.2	4371	3.0	43.3
24	655-2	8.0	23.8	8.6	14.6	1.3	4363	3.3	42.7
25	655-3	8.0	22.8	8.2	13.1	1.2	4357	3.2	45.3
26	655-7	8.3	23.8	8.7	14.7	1.4	4385	3.3	42.4
27	647-6	8.1	23.4	8.6	12.3	1.2	4367	3.2	43.1
28	647-14	8.0	25.5	7.6	13.9	1.2	4390	3.2	40.9
29	647-18	8.2	23.8	8.4	13.5	1.3	4355	3.0	42.7
30	647-21	8.1	24.1	8.5	14.2	1.1	4355	3.1	43.2
31	653-3	8.3	22.7	7.7	12.5	1.3	4369	3.0	42.4
32	653-8	8.2	23.4	8.3	16.2	1.4	4380	3.1	43.2
33	634-2	8.1	24.9	8.7	17.8	1.3	4384	3.2	40.9
34	597-10	8.1	25.4	7.9	16.7	1.2	4397	3.1	39.1
35	597-11	8.1	24.7	8.3	15.1	1.3	4385	3.1	40.3
36	585-4	8.4	25.4	7.6	14.3	1.2	4405	3.1	40.6
	Mean		24.0	8.2	14.2	1.2	4372	3.1	42.4
	Range		22.6-26.8	7.5-9.2	12.3-17.8	1.1-1.5	4340-4406	2.9-3.3	39.1-45.3

Table 2. Wet chemistry analysis, expressed on a dry weight basis, of entries in the Crop Development Centre 2001 Pea Elite Yield Trial (mean data from trials grown at Saskatoon, Rosthern, Elrose, and Outlook)

Entry	Variety	% Moisture	% Cr. pro	% ADF	% NDF	% Eth. Ext.	% Ash	% Starch
1	CDC MOZART	8.2	24.0	7.4	13.1	1.2	2.8	43.0
2	ALFETTA	8.1	23.9	6.8	11.6	1.4	2.7	46.8
3	NITOUCHE	8.0	25.0	7.3	11.9	1.2	2.8	43.6
4	203PMR-16	8.1	27.1	8.5	12.8	1.3	2.9	38.9
5	617-20	8.0	25.2	8.8	13.5	1.1	2.9	40.5
6	CDC0007	8.1	25.3	7.0	13.0	1.0	2.8	42.7
7	CDC0009	8.1	26.1	6.6	11.7	1.2	2.7	41.0
8	CDC0001	7.9	26.9	7.1	11.5	1.2	2.7	42.0
9	CDC0101	8.1	24.6	7.3	13.1	1.2	2.6	41.3
10	511-2	8.1	22.9	7.4	12.9	1.3	2.8	43.4
11	468-33	8.1	25.9	7.3	12.7	1.1	2.8	39.7
12	679-8	8.1	25.0	6.6	12.9	1.1	2.8	41.0
13	CDC0102	8.2	24.3	7.2	12.2	1.2	2.7	46.4
14	635-15	8.1	24.8	6.9	11.7	1.2	2.7	44.3
15	CDC0103	8.1	24.6	6.6	12.4	1.4	2.7	44.9
16	653-8	8.2	24.6	7.2	12.5	1.2	2.7	43.0
17	CDC0104	8.0	24.8	7.1	13.6	1.2	2.7	41.7
18	CDC0105	8.1	25.7	7.0	12.1	1.2	2.7	42.3
19	684-10	8.2	24.3	6.9	12.3	1.1	2.7	44.9
20	429-4	8.0	25.6	7.3	12.4	1.3	2.8	45.1
21	482-4	8.1	25.6	7.3	13.7	1.3	2.8	42.7
22	501-15-2	7.9	25.1	7.5	12.2	1.4	2.8	40.6
23	CDC0106	8.0	24.2	8.2	15.4	1.3	2.9	43.8
24	597-3	8.0	24.4	7.3	12.5	1.3	2.7	40.5
25	603-1	7.9	25.5	7.0	11.0	1.2	2.9	40.3
26	CDC0107	8.0	25.3	7.5	12.8	1.4	2.6	40.3
27	679-10	8.1	23.7	7.2	13.9	1.3	2.8	42.9
28	CDC0108	8.1	25.2	6.7	12.5	1.2	2.7	43.2
29	CDC0109	8.2	25.6	7.4	13.2	1.1	2.9	42.2
30	F2-37=SB2000-1	8.1	24.2	7.8	12.6	1.2	2.7	42.6
31	F13-10=SB2000-3	8.1	25.3	6.9	12.9	1.1	2.8	40.9
32	F6-6=SB2000-2	8.2	24.8	7.6	12.8	1.1	2.8	40.9
33	CDC0004	8.1	24.8	7.4	12.1	1.2	2.7	43.2
34	CDC0010	8.2	25.7	6.3	8.9	1.0	2.9	43.1
35	CDC0011	8.1	26.4	7.3	12.0	1.1	2.7	43.1
36	CDC0110	8.0	25.5	7.3	14.0	1.2	2.8	43.2
	Mean		25.1	7.3	12.6	1.2	2.8	42.5
	Range		22.9-27.1	6.3-8.8	11.1-16.0	1.0-1.4	2.6-2.9	38.9-46.8

Conclusions:

1. Field pea samples tested displayed substantial ranges in mean values for all chemical parameters assessed (starch, protein, acid detergent fiber, neutral detergent fiber, ether extract, and ash). These ranges will be useful in breeding superior feed pea varieties.

2. Several advanced lines were superior to the best check variety in two or more key traits (crude protein, ADF, NDF, or starch) related to feed value, without being inferior in any key trait.

3. NIR has the potential to rapidly assess field pea lines for chemical traits related to feed value.

Papers/Presentations:

Racz, V.J., Warkentin, T.D. and Arganosa, G. (2002) Nutrient parameters and selection for superior feed peas. H-H Muendel (ed.), 4th Canadian Pulse Research Workshop (Edmonton).

Warkentin, T., Vandenberg, A., Arganosa, G., Maenz, D., Racz, V., and Zijlstra, R. (2000) Breeding feed peas for Saskatchewan. Soils and Crops 2000. Extension Division, University of Saskatchewan (Saskatoon).

Information of benefit to producers, processors, or governments:

Improved NIR calibrations for predicting the chemical constituents of feed pea samples will assist pea breeders in selecting varieties with improved protein content/energy content. The calibration equations developed over the course of this project will also be transferred to remote instruments for use in the feed industry.

(d) **Personnel:**

Wet chemistry analysis was conducted at the Prairie Feed Resource Centre under the supervision of Vern Racz. Gene Arganosa at the Crop Development Centre conducted NIR scans.

(e) Equipment:

No equipment was purchased. NIR scans were conducted using equipment in Dr. Brian Rossnagel's laboratory.

(f) Project Developed Materials:
None

(g) Project Photos:
None

(h) Acknowledgement:
The Saskatchewan Agricultural Development Fund has been acknowledged in all presentations where this research project was mentioned.